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Lack of effect of ethylene glycol on transcription of Neurorporo conidial DNA

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Abstract

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Dutta and Chaudhuri (1974 Genetics 77: s19) reported that only 15% of the DNA of untreated Neurorporo conidio is transcribed into RNA, whereas an increase in transcription (25%) occurs when conidia germinate. Ethylene glycol-treated conidio show rignificont changer in growth; they enlarge, gain weight and become osmotically sensitive (Wilson and Bates 1972 Neurorporo Newsletter 19: 21). It was thought desirable to know whether this chemical treatment of conidio changes their pattern of transcription.

N. crassa 74A wild type strain was used throughout this study. Conidia were grown on Vogel's agar medium, washed with distilled water and strained through four layers of cheese cloth. Ethylene glycol treatment of conidial cells was carried out as described by Wilson and Bates (1972 NN#19:21). Conidia were collected by centrifugation at 2000 x g for 20 minutes. Total cellular RNA was extracted according to the method of Kohne and Byers (1973 Biochemistry 13: 2373). Ethylene glycol-treated and untreated conidial cells were broken by passing through a needle valve with a pressure drop of 20,000 and 30,000 psi respectively. Unlabeled DNA was isolated from treated and untreated conidial cells and ^{32}P-labeled DNA was extracted from mycelia as described by Dutta (1973 Biochim. Biophyr. Acta, 324: 482).

^{32}P-labeled DNA and total cellular RNA were denatured at 80° C in the presence of 50% formamide and incubated at 35° C with 0.4M phosphate buffer (PB), 0.6M NaCl, 0.01M EDTA pH 7.0. Less than 2 ug ^{32}P-labeled DNA and a considerable excess of RNA were used so that any hybridization reaction would occur mainly between DNA and complementary RNA and not between complementary DNA strands. After incubation, the annealing solution was passed through a hydroxyapatite column previously equilibrated at 60° C with 0.14M PB pH 6.8 and 0.4% sodium lauryl sulphate. To test whether or not true DNA:RNA hybrid formation had occurred, the procedure described by Dutta (1973 Biochim. Biophyr. Acta, 324: 482) was followed. Table 1 summarizes the extent of hybridization of ^{32}P-labeled non-repeated DNA from mycelia with RNA from treated and untreated conidio. 15-17% of the ^{32}P-labeled mycelial was DNA hybridized with the total RNA of conidio (treated or untreated). There was no rignificont difference in the T_e50 (i.e., the temperature at which 50% of hybrid molecules remain double stranded) values of DNA:RNA hybrids using RNA from treated or from untreated conidia.

Table 1. Transcription of the non-repeated DNA of untreated and ethylene glycol-treated conidio.

Cell type	Whole RNA $C_o t$	^{32}P-Unique DNA $C_o t$	Unlabeled total DNA $C_o t$	Per cent DNA absorbed in HAP
A) Conidio	8.8×10^4	0.78	--	15.00
Ethylene glycol treated conidio	8.8×10^4	0.72		17.00
Germinated conidia	8.6×10^4	0.72	--	25.00
B) Conidia		1x10-3	1320	92.00
Ethylene glycol treated conidio		1x10-3	1560	90.00
Germinated conidia	--	1×10^{-2}	1560	91.00
C) Conidia	8.6×10^4	1.2x10-3	--	0.5
Ethylene glycol treated conidia	8.6×10^4	4x10-2	--	0.0
Germinated conidia	8.6×10^4	1x10-2		0.1

It is apparent that ethylene glycol-treated conidia showed transcription of almost the same fraction of DNA as did ungerminated conidia and clearly less than the 25% transcription shown by germinated conidio. Data given in the table suggest that all of the DNA:RNA reactions were complete. The unabsorbed ^{32}P-DNAs from each reaction in (A) did not hybridize further with additional RNA (C) but did hybridize completely (B) with on excess of unlabeled DNA. These results suggest that the amount of transcription in Neurospora conidia is not significantly affected by ethylene glycol treatment.

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